PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ------	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/MX 02/00083	International filing date *(day/month/year)* 22.08.2002	Priority date *(day/month/year)* 22.08.2002
International Patent Classification (IPC) or both national classification and IPC A23L1/33		
Applicant CENTRO DE INVESTIGACION EN ALIMENTACION etc.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 17 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.06.2004	23.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Vernier, F Telephone No. +49 89 2399-8646

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/MX 02/00083

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-11 as originally filed

Claims, Numbers

1-9 as originally filed

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item I

1. The amendments filed with fax dated 08.12.2004 contain subject-matter which extends beyond the content of the application as filed (Article 33(2)(b) PCT). In particular, there is no disclosure in the application as filed for the temperature range 35-39 °C, pressure range 1275-345 bar and volume range 1875-3200 L of claim 1 filed with said fax. Furthermore, a humidity range of 1-5% is disclosed in relation with the process parameters of claim 4 as filed with fax dated 08.12.2004, and nor 1-10% as indicated in claim 1.
Thus, this report is drafted as if such amendments had not been made.

Re Item V

2. Reference is made to the following documents:
   - D1: CLARKE A.D.: 'Reduction of cholesterol levels in meat, poultry and fish products. En production and processing of healthy meat, poultry and fish products' ADVANCES IN MEAT RESEARCH vol. 11, 1997, (PEARSON M.A. AND DUTSON T.R. ED.), pages 101 - 117
   - D2: EP-A-0 356 165
   - D3: US-A-5 024 846A
   - D4: US-A-5 061 505A
   - D5: YAMAGUCHI K. ET AL.: 'Supercritical carbon dioxide extraction of oils from antarctic krill' J. AGRIC. FOOD CHEM. vol. 34, 1986, pages 904 - 907
   - D6: HARDARDOTTIR L.Y., KINSELLA J.: 'Extraction of lipid and cholesterol from fish muscle with supercritical fluids' JOURNAL OF FOOD SCIENCE vol. 53, 1988, pages 1656 - 1661

3. The subject-matter of present independant claims meets the novelty requirement (Article 33(2) PCT), since none of the cited prior art discloses a low-cholesterol shrimp or a method for producing it.

4. However, in view of the technical problem to be solved (to provide a low-cholesterol shrimp), the subject-matter of present independant claims cannot be regarded as involving an inventive step (Article 33(3) PCT) over any of the closest prior art documents D1, D2, D5 and D7, which all disclose the use of supercritical extraction to obtain low-cholesterol meats. In addition, D2 mentions a step of dehydrating before extraction.

5. Present claim 1 lacks clarity (Article 6 PCT), since it is not clear what is to be understood under the term "edible portion".

LOW-CHOLESTEROL SHRIMP AND METHOD OF OBTAINING SAME

BACKGROUND OF THE INVENTION

Field of the invention

This invention refers to a low-cholesterol shrimp and method of obtaining the same by supercritical fluid extraction (SFE) with supercritical carbon dioxide, which can be marketed for human consumption.

Prior Art

It is known that dietary cholesterol constitutes a significant risk factor in the development of heart disease (Grundy et al., 1982). Which result in a need to reduce the Cholesterol content in proteinic foods. A low-cholesterol shrimp would be a high value-added product for the shrimp fishery and farming industries and at the same time significantly contribute to meet the rising demand for low-cholesterol foods.

Currently, there is considerable interest in reducing the cholesterol content of foods for marketing purposes (Hardardottir and Kinsella, 1988).

Among the various industrial techniques currently used for reducing the cholesterol content of foods is the process known as supercritical extraction which has found various commercial applications. This process relies on the use of a supercritical fluid, e.g. a fluid heated above its critical temperature and compressed above its critical pressure. In the supercritical state, the physicochemical differences between the liquid and the gaseous phases disappear and thus, the fluid can no longer be liquefied with an increment in pressure thus becoming denser (Sihvonen et al., 1999). In this state the fluid has very peculiar thermodynamic and transport properties. Its density is relatively high, similar to that of a liquid which provides high solvent capacity while its low viscosity and high diffusivity, similar to those of a gas, provide a large penetration capacity within the sample. Due to all these properties, the speed for solute mass transfer is larger within a supercritical fluid than within a liquid (Rizvi et al., 1986). By manipulating the operating conditions the supercritical fluid has the ability to selectively extract one or more specific components. such as fats, oils, cholesterol,

ketones, aldehydes and esters while leaving proteins, sugars, and other carbohydrates practically untouched (Dziezak, 1986). The most widely-used supercritical solvent in the food industry today is carbon dioxide because it possesses overwhelming advantages over other compounds. It is non-flamable, non-corrosive, non-toxic and non-pollutant. It is rather inexpensive and its critical temperature is also low (31,1°C). This makes it very adequate for the extraction of thermally-unstable materials.

The fundamental principles for this technology were recognized by Hannay and Hogarth more than 100 years ago, in 1879 (Yamaguchi et al., 1986). Very few processes based on supercritical extraction have been implemented in the food industry with the aim of reducing the fat and cholesterol content of foods from animal origin. According to the review by Clarke (1997), the application of supercritical fluid extraction (SFE) in fresh meat products has had little success in cholesterol reduction. A few successful cases have been achieved with ground beef.

Cully et al. (1991), German Patent Application No. DE-39-29-551-A1 and the US patent No. 5,061,505 describe SFE methodologies with the use of adsorption solids agents. This invention is successful with powdered egg yolk and with butterfat and also with other homogenized or ground foods. However, their invention does not mention whole foods.

The applications of this technology to seafoods are more limited (Yamaguchi et al., 1986). Hardardottir and Kinsella (1988), carried out successful lipid and cholesterol in ground trout muscle. However, the solubility of muscle proteins was reduced due to the extraction. The protein preparations had poor emulsifying properties and did not form gels. These authors did not experiment with whole trout pieces either, only with ground trout.

Up until this day, no process has been proposed for the industrial production of shrimp with a reduced content of cholesterol applying SFE or any other type of processing. Yamaguchi et al. (1986), in his experimental work used a krill species (similar to shrimp but much smaller in size) which was previously ground. However, the shrimp market to a large extent is based on the marketing of whole headless shrimp (shrimp tails). Additionally, in the results obtained with krill, Yamaguchi et al. (1986), reported cholesterol as one of the minor extraction

components. The krill meat deteriorated due to oxidation or depolymerization.

McLachlan, et al. (1990), EPO356165, established a procedure for the extraction of esterols and lipidic components (for example, cholesterol and fat) from high-protein foods such as meat using fluids in a subcritical and supercritical state. This procedure involves an initial treatment of the product to remove all the free water, but not the total bound water. Thus, the process yields an intermediate-moisture product. The removal of moisture is carried out through freeze-drying until the final content is from 30-55%. Supercritical carbon dioxide was used for the removal of lipids immediately separating the fraction of carbon dioxide-fat using a selective adsorbent. The product is later reconstituted using water and fat.

While it is true that a growing interest in research using supercritical fluids has occurred, no treatments have been reported in animal tissues maintaining their original shape. For example, in Patent EPO356165, McLachlan et al. (1990), used food chunks as their sample and included an initial processing stage for size reduction.

While differing from all the research projects and patents published so far, including those of Hasegawa et al. (1984), McLachlan et al. (1990, 1991a, 1991b, 1992) and Cully et al. (1991), and in spite of the fact that cholesterol extraction from the intact muscle presents significant technical difficulties due to the fibrous nature of the muscle itself, in the production of shrimp with reduced cholesterol, which is the object of this invention, a process was developed so that it maintains the original size of the food in order to sep the geometric appearance of the final product, thus respecting its original shape to satisfy the demand of the consumer.

For this reason also, different shrimp species and sizes having high consumer acceptance due to their flavor and shape were included. This situation posed additional problems such as a higher cholesterol content compared to alternate species and a relatively higher size which makes cholesterol removal more difficult.

In the state of the art review presented by McLachlan, et al. (1990, 1991a, 1991b, 1992) several cholesterol extraction procedures for foods are mentioned, including stages of dehydration, cholesterol removal using SFE and product reconstitution. However, the methodologies and specific operating conditions differ from the ones reported in this invention because they are not applicable to shrimp

tissues since these are comprised of shorter muscle fibers which allow faster and easier cooking procedures as compared with the foods reported in the referenced works. Also, shrimp proteins are subject to a quicker deterioration due to their sensitivity to high or low temperatures, chemical agents, physical abuse, etc. and thus require a significantly more careful handling during processing.

In the state of the art review previously mentioned, several methodologies or specific cholesterol SFE operating conditions and product reconstitution procedures are followed which are not applicable to whole pieces of shrimp or to shrimp tissue, since they require more careful handling during processing. The shrimp protein (myosin) is more sensitive to denaturation (freezing, dehydration, cooking) as compared to that of beef, pork or poultry (warm-blooded animals). Also, the molecular characteristics of shrimp make it more susceptible to deterioration due to the action of proteolytic enzymes as compared to the collagen found in higher animals (Crawford, 1981).

SUMARY OF THE INVENTION

Consequently, the first purpose of the invention is to provide a low-cholesterol whole shrimp.

A second object of this invention is to provide a process to obtain a low-cholesterol whole shrimp by a supercritical fluid extraction.

Another object of the invention is to provide a process for producing low-cholesterol whole shrimp, using a supercritical fluid extraction technique comprising a rehydration step which renders a shrimp having suitable sensorial properties which maintain its suitable nutritional properties. Namely, a shrimp with a low level of fat and a high level of proteins.

Still other object of the invention is to provide a freeze drying process which helps to preserve the shrimp structure when it is subject to a structurally detrimental Supercritical fluid extraction (SFE).

The aims of the invention are achieved by a process for obtaining low-cholesterol whole shrimp comprising: (a) to provide a plurality of frozen, peeled and deheaded shrimps; to freeze drying the whole shrimp to a humidity content of

approximately 1 to 10%; (c) to extract the cholesterol from the dehydrated shrimp by a stream of a supercritical solvent which is selective to cholesterol, at a temperature between 35-39°C, at a pressure between 1275-345 bar, and a supercritical solvent volume between 1875-3200 L; (d) to rehydrate the shrimp in a vacuum chamber with water in a relationship of about 1-10mL per g shrimp at vacuum and room temperature by about 1-5 hours; and (e) to cook the whole shrimp with steam.

DESCRIPTION OF THE INVENTION

Shrimp, known by different common names throughout the world, are high-cholesterol foods (Oliveira and Silva, et al., 1997).The present invention refers to a low-cholesterol shrimp obtained from any of the species from the Subgenus *Litopenaeus* (i.e. *L. occidentalis, L. schmitti, L. setiferus, L. stylirostris, L. vannamei*). The procedure is also applicable to other genus, subgenus and species of non-grounded shrimp and to their different sizes, i.e. U-10, U-12, U-15, 16-20, 21-25, 26-30, 31-35, 31/40, 36-40, 41-50, 51-60, 61-70, 71-80 and over 80. Peled, headless whole shrimp is used as raw material. A natural variability in the content of cholesterol is present among the different shrimp species. However, the process described in this invention will work equally well with only minor adjustments in the processing variables, particularly the volume of supercritical fluid used and temperature of extraction.

In accordance with the present invention, the process to obtain a low-cholesterol whole shrimp consists of dehydrating the shrimp and subjecting it to supercritical extraction using a supercritical fluid until the cholesterol content is reduced to a desirable concentration and after that, be rehydrated and cooked.

The first step of the process of the invention comprises a shrimp dehydration aimed at promoting the establishment of intramuscular channels in the food matrix to facilitate the subsequent extraction of cholesterol by allowing the proper circulation of the extracting fluid throughout the tissues. On the other hand, due to the highly perishable nature of shrimp, reduction of its moisture content allows a significant reduction of the spoilage rate during its processing and storage.

In the process reported in this invention the shrimp moisture content was lowered to only 1-10%, thus significantly differing from the 25 to 60% established in the methodology reported by McLachlan et al. (1990), where the free water is removed and only part of the bound water in the food is removed to obtain an intermediate moisture product. Processing shrimp with a water content of 25% or more as McLachlan et al. (1990) report, would result in a non-acceptable product. During the development of the present invention – in which the water content is reduced to 1-10% - twenty freeze-drying experimental trials were performed which took from 12-72 hours, under different operating conditions until a set of conditions were found to provide the such a low water content and at the same time prevent the denaturation of shrimp proteins (this would liberate astaxanthin, the natural muscle pigment in shrimp and form caroteno-protein complexes that give blue, greenish or purple discolorations indicating cooking).

As described in McLachlan et al. (1990), an intermediate moisture product avoids adverse effects on the sensory evaluation properties of the final product which are common in foods subjected to severe dehydration procedures resulting in a water content of less than 15%. In the present invention the final water content of the food was between 1-10% but this condition did not result in rejection by a trained sensory evaluation panel once it was reconstituted. This occurred because once the cholesterol extraction was performed.

A careful methodology was developed in the subsequent reconstitution and storage steps to maintain all aspects of sensory quality at a very high level.

During shrimp reconstitution, applying the traditional methodology for shrimp rehydration (which consists in immersing shrimp in an excess quantity of water at room temperature for a period of time) (Moorjani and Dani, 1968), it was not possible to attain an acceptable rehydration index. A mechanically-assisted rehydration process such as that suggested by McLachlan et al. (1990) results adequate for ground beef but obviously not for whole shrimp since it would undergo considerable damage. Due to the above reasons, several methodologies were devised for rehydrating the shrimp and achieving an adequate rehydration index.

All of these rehydration procedures were evaluated through sensory tests performed on the rehydrated and cooked shrimp. Different cooking procedures were also tested (immersion in boiling water, microwave cooking, steam cooking) until a minimal effect on the sensory properties of shrimp was obtained. The methodology described herein allows the proper rehydration of shrimp with the additional advantage that only water is used for the operation. That is, no polyphosphates, seasoning agents or other ingredients are needed to obtain an acceptable product.

In spite of the fact that a procedure for cholesterol extraction from foods has been reported previously, which comprises the stages of dehydration, cholesterol removal by supercritical extraction and product reconstitution, the methodology and operating conditions differ very significantly from those reported here and they do not apply to shrimp due to its particular geometry, configuration and muscle structure. The processes described in the state of the art are not applicable to whole pieces of shrimp. Shrimp tissue, as is well known, contains more sensitive proteins to deterioration as compared to the tissue of other types of meat.

By applying the process proposed in the current invention, a new product can be obtained, i.e. low-cholesterol shrimp, which has acceptable sensory properties while keeping its nutritional content practically unchanged, i.e. a low fat content (1% or less) and a high protein content (15-20%).

For obtaining the low-cholesterol whole shrimp of the present invention, frozen peeled deheaded shrimp are used. The method of the invention comprises an initial dehydration step. The dehydrated shrimp immediately pass to the next processing step which consists of supercritical extraction of cholesterol using a highly selective supercritical solvent for lipids at a nominal pressure and temperature. For this purpose, supercritical extraction equipment with carbon dioxide as the supercritical fluid is utilized. The dehydrated shrimp are placed in the equipment extraction unit and carbon dioxide is compressed above its critical pressure (100-400 bar). The resulting gas enters the extraction unit supplied with a heating jacket to allow the extraction temperature to be maintained in a range from 30-60°C and pass through the sample to remove the cholesterol. This process can be applied to any shrimp species and sizes with slight variations in the operating conditions. The discharge gas containing the extract is then passed through an

expansion valve. At this point the extract is released from the gas by precipitation since a pressure differential under supercritical conditions implies a decrease in density and a lowering of the solvating capacity.

Dehydrated shrimp containing lower cholesterol content are thus obtained and reconstituted with water using a ratio of 1 to 10 mL per gram of shrimp. Rehydration is carried out by placing shrimp in a vacuum chamber at room temperature for a period of 1-5 hours. The dehydrated shrimp is steam-cooked before presentation to the final consumer in its original shape.

The final product obtained through this process is a low-cholesterol whole shrimp which is a non-existent product up to the time of this invention. Additionally, this product complies with all the nutritional labeling requirements established for low and reduced-cholesterol products by the Food and Drug Administration. According to such requirements a cholesterol-reduced product must contain 75% or less cholesterol than the original food from which it is obtained, and for a low-cholesterol product it must contain from 2 to 20 mg of cholesterol per serving (FDA, 1986). A standard error of 20% is allowable in these levels and therefore a cholesterol content of less than 24 mg per serving is acceptable for low cholesterol foods (FDA, 1990).

The final form of the product, subject of the present invention, consists of whole pieces of shrimp, not in chunks, or dices or powder, as has been the case with other products processed by supercritical extraction. Also, the shrimp continues to possess its original sensory characteristics in terms of texture, flavor, color and overall appearance. A sensory test was performed to evaluate each of these attributes on dehydrated and supercritically-extracted shrimp after rehydration and cooking. Such shrimp corresponded to the category of Low-cholesterol shrimp (less than 24 mg of cholesterol per serving). The test was applied to a panel of 30 untrained subjects each of whom evaluated sample color, odor, and overall appearance. The evaluation score was from –3 a +3. For a statistical analysis a non-parametric Kolmogorov-Smimov test was performed. All attributes evaluated had a positive score from the panel members and no significant differences were evident in the acceptance scale. The texture and flavor attributes had a moderate acceptance score (+2) while smell, color and overall appearance gave a slight acceptance score (+1) (Fig. 1). In relation to smell and

taste, several panelists observed that a favorable condition had been caused by the supercritical extraction process since it diminished the typically-strong smell of the product. These results show promising perspectives for the overall acceptance of low-cholesterol shrimp by the end consumer.

The following example is used to illustrate the novelty and utility of the present invention, without intention to limit the scope of the invention.

EXAMPLE

The raw material used was "blue shrimp" (*Litopenaeus stylirostris*) and "white shrimp" (*Litopenaeus vannamei*), 16-20 count per pound and deheaded, which were kept under frozen storage (-18°C) until processed.

Shrimp are thawed, peeled and individually refrozen at –40°C for a period of 4 hours using a quick freezing system. The shrimp are then freeze-dried until a final water content of 1-5% is reached. The temperature on the products surface as well as that inside is carefully monitored using thermocouples. When the equipment reaches a 0,1 mm Hg vacuum the following program of conditions is to be followed:

Temperature °C	Time hs
-29	1
0	1
50	4-5 [a]
35	15-20 [b]
25	1-3 [c]

[a] The time will depend on the level of vacuum achieved, which should not exceed 0,2 mm Hg.

[b] The time will depend on when the shrimp reach a maximum temperature of 5 to 10 °C.

[c] Depending on when the internal shrimp temperature becomes the same as that on their surface.

Once the freeze drying process is completed, the shrimp are ready for the extraction of cholesterol.

Cholesterol extraction is carried out in a supercritical extractor using a selective solvent (carbon dioxide) under supercritical conditions of 310 bar and 37°C. For this purpose supercritical extraction equipment with carbon dioxide is used. The extraction system consists of four basic components: a compressor or solvent pump, an extractor, a control system for pressure and temperature and a separator.

Once the shrimp have been placed within the extraction vessel the carbon dioxide is allowed to flow from the storage tank and through the compressor in order to attain the supercritical pressure of 310 bar. The resulting gas enters the extraction chamber containing a heated jacket which allows the operating temperature to be maintained at 37°C. Upon contact of carbon dioxide with the shrimp sample, a process of selective extraction begins and the gaseous carbon dioxide picks up the free cholesterol in the shrimp tissue and produces a cholesterol-rich extract. Both the volume of carbon dioxide and the flow speed are measured carefully. The flow speed should be maintained at 5,5-6,2 L/min but other flow velocities can also be used. After the super extraction step, cholesterol is separated from the carbon dioxide by means of an expansion valve.

The super extraction step concludes when 1875 L of supercritical Carbon dioxide are introduced in the system. The spent carbon dioxide can be recycled to the system.

Once the supercritical extraction process is completed, the shrimp are subjected to rehydration using water at room temperature in a relationship of 5 mL of water per gram of shrimp. This process should take place under vacuum (533 mm Hg) for at least one hour. At the end of this period the shrimp is turned on its side and allowed to rehydrate under the same conditions for one more hour.

Upon rehydration, shrimp can be steam-cooked and later packaged in plastic containers under vacuum and quickly-frozen at –40°C.

The final product obtained with this process conditions complies with the requirements set forth by FDA for low cholesterol food products.

EXPERIMENTAL DESIGN

A Surface Response Methodology was followed during the course of experimentation with the aim of determining the optimal conditions for cholesterol removal from shrimp by supercritical extraction. A compounded-central rotatory design was applied for three independent variables with five levels for each one. The number of experimental points in the design was sufficient to prove the statistical validity of the quadratic model obtained (Arteaga et al., 1994). The variables used in the stage of cholesterol extraction were: Pressure (X_1), Volume (X_2) and Temperature (X_3). The minimum and maximum levels of the variables were fixed according to results obtained in preliminary experiments. The response variable (Y) was the amount of cholesterol remaining in the final product (dry weight basis) as determined by Gas Chromatography.

Table I shows the average values of the remaining cholesterol content in the end product and the corresponding extraction index (%).

TABLE I

TREATMENT	X_1 P (bar)	X_2 V (L_{CO2})	X_3 T (°C)	Y CHOLESTEROL (mg/100g) dry basis	CHOLESTEROL (mg per serving) wet basis	EXTRACTION %
1	289	909	36	225,10	52,25	61,99
2	331	909	36	292,71	67,95	50,56
3	289	2841	36	151,41	35,15	74,43
4	331	2841	36	81,96	19,26	85,99
5	289	909	38	224,88	52,20	62,02
6	331	909	38	211,06	49,00	64,35
7	289	2841	38	72,19	16,76	87,81
8	331	2841	38	52,02	12,08	91,21
9	275	1875	37	114,25	26,52	80,70
10	345	1875	37	99,23	23,03	83,24
11	310	250	37	366,61	85,11	38,08
12	310	3500	37	62,14	14,43	89,50
13	310	1875	35	125,44	29,12	78,81
14	310	1875	39	97,25	22,58	83,57
15	310	1875	37	99,68	23,14	83,16

In order to generate an equation that forecasts the effects of operating conditions (X_1, X_2, X_3) on the cholesterol quantity remaining in the final product, a

regression program was run. By multiple regression analysis a quadratic model was adjusted and a final regression equation calculated:

$$Y = 6065{,}3575 - 0{,}608833\ P + 0{,}1424819\ V - 303{,}0457\ T + 0{,}0147289\ P^2 - 0{,}000884\ VP + 0{,}0000475\ V^2 - 0{,}191346\ TP - 0{,}003532TV + 4{,}7773254\ T^2$$

where:

Y = Remaining cholesterol in final shrimp product (mg/100g) Dry Weight Basis

P = Supercritical extraction pressure (bar)

V = Carbon dioxide volume (L)

T = Supercritical extraction temperature (°C)

The results from the Analysis of Variance for the quadratic model of prediction are presented in Table II wherein a significant effect of the adjusted model ($p \leq 0{,}05$) is evident. Also, the lack of fit resulted non-significant ($p > 0{,}05$). This information supports the validity of the model.

TABLE II

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F-Ratio	p	R^2
Regression	9	121477,4	13497,49	18,31	0,000202	0,953705
Linear effect	3	94030,26	31343,42	42,52	0,000029	0,738221
Quadratic effect	3	24653,4	8217,8	11,15	0,003140	0,193551
Interactions	3	2793,743	931,2475	1,26	0,350317	0,021933
Total error	8	5896,731	737,0914			0,046295
Lack of fit	5	5288,337	1057,667	5,22	0,102269	0,041518
Pure error	3	608,3938	202,7978			0,004776

According to the Analysis of Variance, some linear and quadratic effects were significant ($p \leq 0{,}05$) for the supercritical extraction process from shrimp, with the most important quadratic effect being that of volume.

Fig. 2 shows a Surface Response graph which illustrates the final regression equation obtained through this experimentation. The effect of supercritical extraction operating conditions on the remaining shrimp cholesterol

content on a dry weight basis highlights the effect of different solvent volumes required, according to the final cholesterol content desired.

Fig. 3 shows the amount of remaining cholesterol in the shrimp (on a dry weight basis) as a function of temperature at different volumes of carbon dioxide and a pressure of 345 bar. It can be observed that at such pressure the quantity of remaining cholesterol decreases with an increase in the volume of carbon dioxide with respect to temperature. In a supercritical fluid, the effect of temperature on solubility is quite complex due to two concurrent effects. One effect tends to increase solubility with an increase in temperature, while the other tends to decrease it. As the temperature increases, the solute vapor pressure also increases and this increases solubility. On the other hand, density decreases and this tends to decrease solubility. In this experimental region density is less sensitive to temperature changes and the vapor pressure is the dominant factor so that increases in temperature increase solubility. The temperature at which a minimum of cholesterol content remains is 39 °C (11,77 mg/100 g, dry weight basis). Nevertheless the remaining amount of cholesterol (100 mg per 100 g of shrimp, on a dry weight basis) is sufficient to achieve the FDA requirements for a low-cholesterol food product once it has been rehydrated and cooked, i.e. less than 24 mg cholesterol per 100g shrimp serving on a wet basis.

From Fig. 4 it can be observed that with the conditions given in the example provided for this invention the residual cholesterol content attained is 100 mg per 100 g of shrimp on a dry weight basis. It is clear from Figures 3 and 4 that with different combinations of operating conditions during supercritical extraction, the same result is achieved. The conditions given are less drastic so that their adverse effects on the sensory properties of the final low-cholesterol shrimp product are significantly decreased.

Some advantages of the low-cholesterol whole shrimp are the following:
The low-cholesterol whole shrimp contains less cholesterol than its natural counterpart which is considered a high cholesterol food according to the requirements set forth by the Food and Drug Administration for reduced-cholesterol products (75% or less cholesterol than the natural product). and low-cholesterol (less than 24 mg of cholesterol per 100g shrimp serving on a wet basis).

The low-cholesterol whole shrimp is adequate for human consumption, with the same nutritional properties as the natural product, i.e., a protein content from 15 to 25% and a fat content of less than 1%: a mineral content of 1-3% and a moisture content of 50-80%, which possesses sensory and overall sensory properties acceptable to the consumer

The shrimp of the present invention is a ready-to-eat product as well as a flavor enhancer or its use in salads or other prepared dishes.

The product of the present invention is not affected in its high protein and low fat content and presents sensory properties acceptable to the final consumer.

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CLAIMS

1. A process for obtaining a low-cholesterol whole shrimp comprising:

a. to provide a plurality of frozen, peeled and deheaded shrimps;

b. to freeze drying the whole shrimps to a humidity content of approximately 1 to 10%;

c. to extract the cholesterol from the dehydrated shrimps by means of a stream of a supercritical solvent which is selective to lipids, at a temperature between 35-39°C, at a pressure between 1275-345 bar, and a supercritical solvent volume between 1875-3200 L;

d. to rehydrate the shrimps in a vacuum chamber with water in a relationship of about 1-10mL per g shrimp at vacuum and room temperature by about 1-5 hours; and

e. to cook said plurality of whole shrimp with steam.

2. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that the shrimp is selected from U-10, U-12, U-15, 16-20, 21-25, 26-30, 31-35, 31/40, 36-40, 41-50, 51-60, 61-70, 71-80 and over 80, preferably size16/20.

3. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that the dehydration of shrimp is performed by freeze drying.

4. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that of dehydrating the shrimps, is conducted in a freeze-drier chamber which is initially set at a temperature of -40 °C in which the equipment reaches a vacuum of 0,1 mm Hg and the following set of conditions is applied:

Temperature	Time
°C	hs
-29	1
0	1

50	4-5
35	15-20
25	1-3

5. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that shrimps are dehydrated to a humidity content between 1 to 5%.

6. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that the supercritical solvent is CO_2 supercritical.

7. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that supercritical solvent is CO_2 supercritical at 310 bar pressure and 37°C temperature.

8. The process for obtaining low-cholesterol whole shrimp according to claim 1, characterized in that shrimps are rehydrated under a vacuum at least of 533,4 mm Hg.

9. The low-cholesterol whole shrimp obtained by the process of any of claims 1 to 8.